GE Healthcare
Life Sciences

BioPilot™ Column Mono Q™ 60/100

User Manual

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1 Introduction

BioPilot Column Mono Q 60/100 are prepacked ion exchange columns for high performance separation of proteins and peptides at pilot and small volume production scale. They are intended for operation with BioPilot System.

This column contains a titanium frit in the adapter distribution unit and bottom piece.

After cleaning-in-place (CIP) procedures using acidic or basic solutions, wash the column with two column volumes of neutral buffer before final re-equilibration or storage in 20% ethanol.

If more extensive washing of the frit is required, remove the o-ring from the adapter distribution unit or bottom piece and place the unit or bottom piece (complete with mounted frit only) in the cleaning solution.

The following instructions should be used as guidelines when handling BioPilot Columns to ensure trouble-free use and maximum column life length.

2 Unpacking

When unpacking your BioPilot Column carefully check the contents of the box against the following list.

Item	Code no.
BioPilot Column Mono Q 60/100	17-1002-01

Contents

BioPilot Column Mono Q 60/100

Quality control test sheet

Instruction manual

Pressure expansion syringe

Accessory package containing:

- Filter tool 60 mm
- Filter kit Prefilter 6000
- Tubing connector
- Filter tool
- Stop plug
- Wrench (plastic)

3 Description

3.1 Media

Mono Q is a strong anion exchanger based on MonoBeads™, a beaded hydrophilic resin. The functional group on the matrix is the quarternary amino group identical to that used in Q Sepharose™ High Peformance and Q Sepharose Fast Flow (Figure 3.1). The selectivity of Mono Q is analogous to both Q Sepharose High Performance and Q Sepharose Fast Flow. The exclusion limit of Mono Q is approximately equivalent to M_r=10^7 for globular proteins, allowing higher dynamic capacity for both large and small molecular weight proteins. The ionic capacity of Mono Q is 0.27 to 0.37 mmol Cl^-/ml medium. The particle size range is 10 µm ± 0.6 µm.

$-CH_2-N^+(CH_3)_3$

Figure 3.1: Quaternary amine group.

3.2 Column

BioPilot Column 60/100 has an internal diameter of 60 mm and a packed bed height of 100 mm. The volume of the packed bed is 300 ml. All wetted parts of BioPilot Column 60/100 are manufactured from fully biocompatible materials: fluoroplastic, borosilicate glass, polypropylene, polyethylene, ETFE tubing and Titanium.

The pressure limit on BioPilot Column Mono Q 60/100 is 2 MPa (20 bar) in the BioPilot system. The maximum flow rate is 94 ml/min (flow velocity 200 cm/h) at room temperature in aqueous buffer. This corresponds to a process capacity of 20 column volumes per hour.

Note: *Flow velocity (cm/min) is calculated by dividing the volumetric flow rate (ml/min) by the cross-sectional area of the column.*

3.3 Chemical stability

BioPilot Column Mono Q 60/100 may be used in aqueous media over the pH range 2 to 12 for continuous operation. It is also designed to withstand the rigorous conditions used for cleaning-in-place (CIP) and process hygiene procedures. (For details see *Section 5.3 Cleaning-in-place (CIP), on page 11*, *Section 5.4 Column hygiene, on page 12* and *Section 6.2 Media, on page 15*. Chaotropic agents (6 M guanidinium hydrochloride, 8 M urea) and detergents (cationic and non-ionic) can be used as well as polar organic solvents such as ethanol. All strong oxidizing agents should be avoided.

3.4 Column properties

Property	Description
Type of exchanger	Strong anionic
Ionic capacity	0.27 to 0.37 mmol/ml
Column volume	300 ml
Max. flow rate	94 ml/min (200 cm/h)
Max. column pressure [1]	2 MPa (20 bar)
Bead size	dp50 = 10 µm
Efficiency	>25,000 N/m
Exclusion limit	M_r=10^7
Chemical stability for CIP	1 M NaOH 1 M acetic acid 8 M urea 6 M guanidinium hydrochloride 30% isopropanol 30% acetonitrile 70% ethanol
pH stability, working range	2 to 12
pH stability, CIP	2 to 14

1 in BioPilot System

4 Installation

BioPilot Column Mono Q 60/100 is designed to connect to BioPilot System.

1 Remove the stop plug from the column inlet and the pressure expansion syringe and tubing from the column outlet.

2 Place the column on the P-6000 pump housing.

3 Connect the column inlet to port **2** of motor valve IMV-8 No. 2, using 1.2 mm i.d. tubing (as provided in the Start-Up Kit with BioPilot System) and the column outlet to port 2 of IMV-8 No. 3.

Up to 7 different columns can be connected to BioPilot System simultaneously.

Note: *When connecting pre-flanged tubing, turn the tubing connector until finger-tight and then use the plastic wrench for a final half turn.*

4.1 Startup and equilibration

The column is delivered in 20% ethanol as an antimicrobial agent. This has to be washed out before the equilibration procedure can be started.

1 Wash away the 20% ethanol using low ionic strength buffer. 1.5 column volumes are needed and the flow velocity should be limited to 50 cm/h (25 ml/min for 60/100 columns). After 1.5 column volumes of buffer, double the flow rate and wash with another 3 column volumes of buffer.

2 Introduce the desired counter ion by washing with high ionic strength elution buffer for 5 column volumes at a flow velocity of 100 cm/h (50 ml/min).

3 Equilibrate the column to starting conditions by passing 5 column volumes of start buffer through the column at a flow velocity of 150 cm/h (70 ml/min). Note the back pressure in the system as registered by the pumps.

BioPilot Column 60/100 should not be exposed to a system back pressure higher than 2 MPa (20 bar).

The column is now ready for use.

4.2 Solvent and raw material preparation

To ensure long column life and trouble-free operation of the BioPilot columns it is essential to take great care in preparing both the buffers and the raw material for chromatography. It is very important to filter all liquids entering the column. All solvents should be degassed by sonication or vacuum filtration and filtered through 0.45 µm filters. When running under sterile conditions, 0.22 µm filtration should be employed. Water should be of highest possible purity. Solvents, salts and buffers should be of analytical or pharmaceutical quality to ensure maximum column life-length.

Recommended buffers are listed in Chapter 8.

The quality and composition of the raw material will directly determine the life time of the column:

- For ion exchange the lipid content should be kept as low as practically possible
- Turbid solutions should not be applied
- Endogenous protein should be fully solubilized
- Particulate matter should be removed by filtration or centrifugation

An efficient way to get maximum protein loading capacity on the BioPilot column is to condition the raw material by transferring the protein solution to the column starting buffer by buffer exchange using Sephadex™ G-25 Medium or Coarse. Low molecular weight charged material such as amino acids and even phospholipids can be removed by this operation.

4.3 Loading capacity

The practical working range of BioPilot Column Mono Q 60/100 is up to 20 mg protein per ml of medium, depending on the starting material, for continuous gradient elution.

The feed volume is not generally regarded as a limiting factor in ion exchange chromatography since feed volumes of up to 20 times the column volume may be applied for continuous gradient elution. The time taken to process large volumes, however, can be limiting. Therefore when handling large volumes of starting material such as hybridoma cell culture supernatants, rapid concentration of the starting material should be considered to reduce total cycle time. This can be performed using Sepharose Fast Flow ion exchange media.

5 Operation

5.1 Regeneration

After each separation cycle the column is regenerated by passing two column volumes of a 1 M solution of the elution buffer salt through the column. This step will remove any proteins that bind strongly to the column by ionic interaction under the conditions employed during separation.

5.2 Equilibration

After regeneration the column is equilibrated for the next separation step by passing at least two column volumes of the start buffer through the column at a flow velocity of 200 cm/h (94 ml/min). A steady UV baseline should always be obtained before the raw material is applied to the column.

5.3 Cleaning-in-place (CIP)

After repeated separation cycles at high protein loads a progressive buildup of contaminating material can occur on the column. This material may not be removed by the regeneration step described above. The nature and degree of contamination will depend on the raw material and the chromatographic conditions employed and these should be considered when designing a cleaning protocol.

The following are CIP protocols which can be used as guidelines to formulate a cleaning protocol specific for the raw material to be applied. The frequency of use will depend on the raw material but it is recommended to use a CIP protocol at least every 10 cycles during normal use. If the nature of the contamination is unknown then protocols 1-3 can be used in sequence separated by a wash of one column volume of water.

After cleaning-in-place (CIP) procedures using acidic or basic solutions, wash the column with two column volumes of neutral buffer before final re-equilibration or storage in 20% ethanol.

Purpose	Procedure
Protocol 1	
Removal of strongly ionically bound protein.	Wash the column with a 1/2 column volume of filtered 2 M NaCl solution at 50 cm/h.
Protocol 2	
Removal of strongly bound hydrophobic proteins or lipoproteins.	Wash the column with 4 column volumes of 1 M NaOH solution at 40 cm/h.

Purpose	Procedure
Protocol 3	
Removal of lipid and very hydrophobic proteins.	Wash the column with 5 column volumes of 70% ethanol at 40 cm/h.

All flow velocities are defined at room temperature.

5.4 Column hygiene

5.4.1 Sterilization

NaOH is an effective cleaning agent for chromatographic media and it has also been shown to be very effective for the destruction of bacteria and yeast cells which could contaminate the media. Tables 5-1 and 5-2 show the relationship between the concentration of NaOH and the inactivation time of Gram negative *Pseudomonas aeruginosa* bacteria and Gram positive *Bacillus subtilis* spores.

Table 5.1: Time required for inactivation of *Pseudomonas aeruginosa* at room temperature.

NaOH conc. (M)	Time (h)
0.15	16
0.25	16
0.5	5
1.0	4

Data from Dr. F. Hasko, Institute of Haematology, Budapest, Hungary.

Table 5.2: Time required for 99% inactivation of *Bacillus subtilis* spores at different temperatures.

NaOH conc. (M)	4°C	20°C
0.5	76 h	8 h
1.0	22 h	2 h

Data from Whitehouse R.L., Clegg, L.F.L., *Journal of Dairy Research* **30**. 315-322 (1963).

Note: *The efficiency of NaOH is both time and concentration dependent. For efficient sanitation, 0.5 M NaOH should be used and the sanitation should preferably be carried out at room temperature to minimize the contact time required.*

Mono Q is well suited to these sanitizing conditions.

5.4.2 Decontamination

Inactivation of biologically active material can be divided into two categories:

1 Inactivation of viruses.

2 Inactivation of contaminating microbial products.

Certain detergents, organic solvents and NaOH have been shown to be effective inactivating agents for different viruses that may originate from mammalian cell culture, tissue extracts and blood plasma [1] [2]. Martin et al.[2] have shown that 0.1 M NaOH at room temperature inactivates the HTLV-III/LAV virus in 10 minutes.

Inactivation of biologically active bacterial products (typically endotoxins in the form of lipopolysaccharides) can prove to be a problem. *E. coli* endotoxin has been shown to be heat resistant[3]. Investigations at GE Healthcare have shown that NaOH is effective for inactivating *E. coli* endotoxin (Figure). The inactivation efficiency is highly dependent on NaOH concentration.

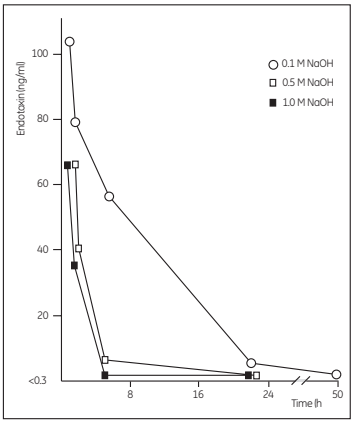

Figure 5.1: Inactivation of E. coli endotoxin in alkaline environment at room temperature.

Further information concerning endotoxin inactivation can be obtained from chapters 1 and 8 in reference no. 3, the PDA Technical Report No. 7.

1 Inactivation of Hepatitis B and Hutchinson Strain NonA-, NonB-Hepatitis viruses by exposure to Tween 80 and ether. Prince, A. M., Horowitz, B., Brotman, B. *et al.*, *Vox Sang.* **46**. 361-43 (1984).

2 Disinfection and inactivation of the human T lymphotropic virus type III/lymphoadenopathy-associated virus. Martin, L. S., McDougal, J. S., Loskowski, S. L., *J. Infect. Diseases* **152**. 400-403 (1984).

3 Parenteral Drug Association Technical Report No. 7 (1985). PDA Inc., Philadelphia, USA.

6 Maintenance

An increase in pressure drop over the column, discoloration of the top of the packed bed or non-reproducible function indicates contamination and/or blockage in the column. The following sections describe recommended procedures for dealing with contamination.

6.1 Column

Blockage of the column can be identified by isolating the column (programme column by-pass in BioPilot System) and checking the back pressure in the system. If an increased back pressure is observed, locate the blockage point in the system by systematically isolating the different components in the line of flow.

If the back pressure increase is due to a blockage in the column, the following procedures should be employed.

6.1.1 Changing the prefilter

The prefilters are located at the column inlet and outlet. Each prefilter is held in place by an upper and lower support screen.

1 Unscrew the top of the prefilter holder and remove the upper support screen and prefilter using the filter tool.

2 Insert a new prefilter, making sure the lower support screen is still in place.

3 Replace the upper support screen and replace the top of the prefilter holder to finger tightness.

It is recommended to change the inlet prefilters regularly.

6.1.2 Changing the adapter distribution unit

Equilibrate the column in 20% ethanol before removing the adapter.

Adapter removal

1 Disconnect the column inlet from the system.

2 Note the height of the packed bed.

3 Remove the column adapter unit by turning the head of the column counter-clockwise until the unit can be lifted out.

Distribution unit replacement

1 Remove the adapter pre-filter top piece and the black securing ring.

2 Lift the adapter pre-filter housing free. A length of preflanged tubing connects the pre-filter housing to the distribution unit at the bottom of the adapter.

3 Use the supplied plastic wrench to disconnect the tubing from the pre-filter housing.

4 Use the supplied filter tool to loosen the distribution filter unit by turning counter-clockwise.

5 Remove the distribution filter unit from the adapter body and replace with the new unit.

6 Tighten the unit with the filter tool.

7 Reconnect the preflanged tubing of the new distribution unit to the pre-filter housing.

8 Use the plastic wrench to tighten a half turn. Replace the pre-filter housing and secure with the black securing ring.

Adapter replacement

1 Fill the adapter with 20% ethanol by connecting to the system and starting the pump at a flow rate of 10 ml/min.

2 Disconnect the adapter. Replace the adapter unit in the column body, making sure the groove in the adapter is in line with the Allen screw in the column body. Turn the head of the column clockwise until the adapter rests on the packed bed. Continue to turn the column head until the bed is compressed as much as possible.

3 Reconnect the column to the system and pump 20% ethanol through the column at 2 MPa (20 bar) back pressure until the level of the bed has dropped 1 mm. Stop the pump and readjust the adapter position. Repeat this procedure until the bed has been compressed to approximately its original height. Finally turn the adapter head 1 turn. Equilibrate the column as described in *Section 4.1 Startup and equilibration, on page 9*.

If more extensive washing of the frit is required, remove the o-ring from the adapter distribution unit or bottom piece and place the unit or bottom piece (complete with mounted frit only) in the cleaning solution.

6.2 Media

If fouling of the media is suspected, specific cleaning procedures should be employed. When the cause of fouling, i.e., the problem-causing material present in the raw material, is known, one or more of the following procedures should prove useful.

6.2.1 Precipitated proteins

Use the procedure employed for the extraction of the protein.

Wash with 1 M NaOH at a flow velocity of 20 cm/h (10 ml/min) for 2 hours, rinse with 1.5 column volumes of water, then elute the solubilized protein with 1 column volume of a strong salt solution e.g., 2 M NaCl.

If the protein is soluble in acetic acid, wash with 1 M acetic acid at a flow velocity of 20 cm/h for 2 hours.

Fill the column with 0.1% pepsin in 0.05 M HCl and leave overnight at room temperature.

6.2.2 Hydrophobic peptides

These are usually soluble in organic solvents e.g., ethanol.

If the percentage organic solvent which best dissolves the contaminant is known, run this overnight. Use low flow velocities (20 cm/h) to conserve solvents as the contact time is most important. If the optimal percentage is not known, wash with saw tooth gradients 0%-100%-0% of organic solvent and water.

6.2.3 Nucleic acids/charged sugars

DNA and RNA are very soluble in solutions of low ionic strength e.g., 10 mM Tris/HCl, 1 mM EDTA, pH 8.0. Prolonged washing (24 hours) with this solution can in most cases resolubilize nucleic acids which have precipitated on the column.

6.2.4 DNA/RNA-chemical fragmentation

A 2 M NaOH wash for 1 hour will hydrolyze both DNA and RNA. Rinse with 1.5 column volume of water and desorb the small DNA/RNA fragments with 1 column volume of 3 M KCl.

6.2.5 Lipids

For lipoproteins or hydrophobic peptides, wash the column with detergent in basic or acidic solution. Use 0.5% non-ionic detergent in 1 M acetic acid. Wash at a flow velocity of 20 cm/h. Residual detergent can be removed by washing with 5 column volumes of 70% ethanol.

Very hydrophobic lipids can be dissolved in non-polar solvents. Rinse with 30% iso-propanol. Then run a gradient from 30% isopropanol to 100% water in 10 column volumes at a flow velocity of 20 cm/h.

All flow velocities are defined at room temperature.

6.3 Testing the column

6.3.1 Efficiency

After media and/or column maintenance procedures, the efficiency of the column should be checked. The efficiency of the column is an indication of column packing and performance. Column efficiency (N) can be estimated using the following equation:

$N/m = 5.54 (V_R/w_h)^2 1000/L$

L = bed height (mm)

V_R = peak retention (elution) volume

w_h = peak width at half peak height

The experimental conditions are described in the Quality Control Test Sheet supplied with the column.

6.3.2 Back pressure

Estimate the back pressure in the column at a given flow rate by subtracting the pressure generated in the system up to the column inlet (A), from the pressure generated by the total system plus column (B). (A) can be measured by disconnecting the pre-flanged tubing from the column inlet and applying the appropriate flow rate.

7 Spare parts and accessories

Table 7.1: Spare parts and accessories.

Item	Code no.
Distribution unit 60 mm	18-4610-60
Filter tool 60 mm	18-4284-01
Filter kit Prefilter 6000	18-4584-01
Tubing connector	18-4274-01
Filter tool	18-1153-20
Stop plug	18-1102-21
Wrench (plastic)	19-7481-01

Spare keys for the BioPilot Column box can be obtained from your local GE Healthcare representative.

8 Choice of buffer

Table gives recommended buffers for various pH intervals based on runs performed in our laboratories. All values were determined at room temperature.

Table 8.1: Buffers recommended for Mono Q on the basis of experiments performed in our laboratories.

pH interval	Substance	Concentration	pKa	Counterion
5.5 to 6.0	L-histidine	20 mM	5.96	Cl^-
5.8 to 6.4	bis-Tris	20 mM	6.46	Cl^-
7.3 to 7.7	triethanolamine	20 mM	7.76	Cl^-
7.6 to 8.0	Tris	20 mM	8.06	Cl^-
8.4 to 8.8	diethanolamine	20 mM	8.88	Cl^-
9.0 to 9.5	ethanolamine	20 mM	9.5	Cl^-

9 Column storage

BioPilot Column 60/100 should be stored in a solution containing an effective bacteriostatic agent. 20% ethanol at neutral pH is recommended.

After cleaning-in-place (CIP) procedures using acidic or basic solutions, wash the column with two column volumes of neutral buffer before storage in 20% ethanol.

10 Quality control test

To maintain high quality standards, the efficiency of each BioPilot Column is tested using the experimental conditions listed in Table 10:1.

Table 10.1: Conditions for column efficiency test

Sample	1.0 ml acetone, 20 mg/ml
Eluent	50% ethanol
Flow velocity (Flow rate)	60 cm/h (30 ml/min)
Detection	280 nm, 2 mm cell, 0.1 AUFS
Chart speed	3 cm/min

The efficiency of the column is calculated using the equation:

$N/m = 5.54 (V_R/w_h)^2 1000/L$

L = bed height (mm)

V_R = peak retention (elution) volume

w_h = peak width at half peak height

The parameters V_R and w_h can be measured as shown below.

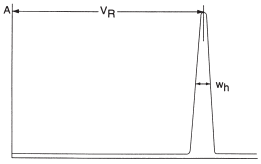

Figure 10.1: Peak retention and peak width parameters.

The efficiency of BioPilot Column Mono Q 60/100 exceeds 25,000 theoretical plates per meter (N/m).

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